

Cell line	Origin	Flow Cytometry
A9 neo	Lung fibroblast L cells	-
A9-m5T4	Lung fibroblast L cells	++++
B16 F10 Neo	Melanoma	-
B16 F10-m5T4	Melanoma	++
EMT6	Mammary adrenocarcinoma	+++
C127 I	Mammary carcinoma	+++
Clone M3	Melanoma	-
EL4	Lymphoma	-
KLN-205	Squamous cell lung carcinoma	+/-
JC	Breast adenocarcinoma	-
LL/2	C57BL Lewis lung carcinoma	-
Mosec	Ovarian carcinoma*	-
Nulli 2A	Embryonal carcinoma	+
129 ES	Embryonic stem cell	-
CL-S1	BALB/c mammary pre-neoplastic alveolar nodules	+/-

Table 1

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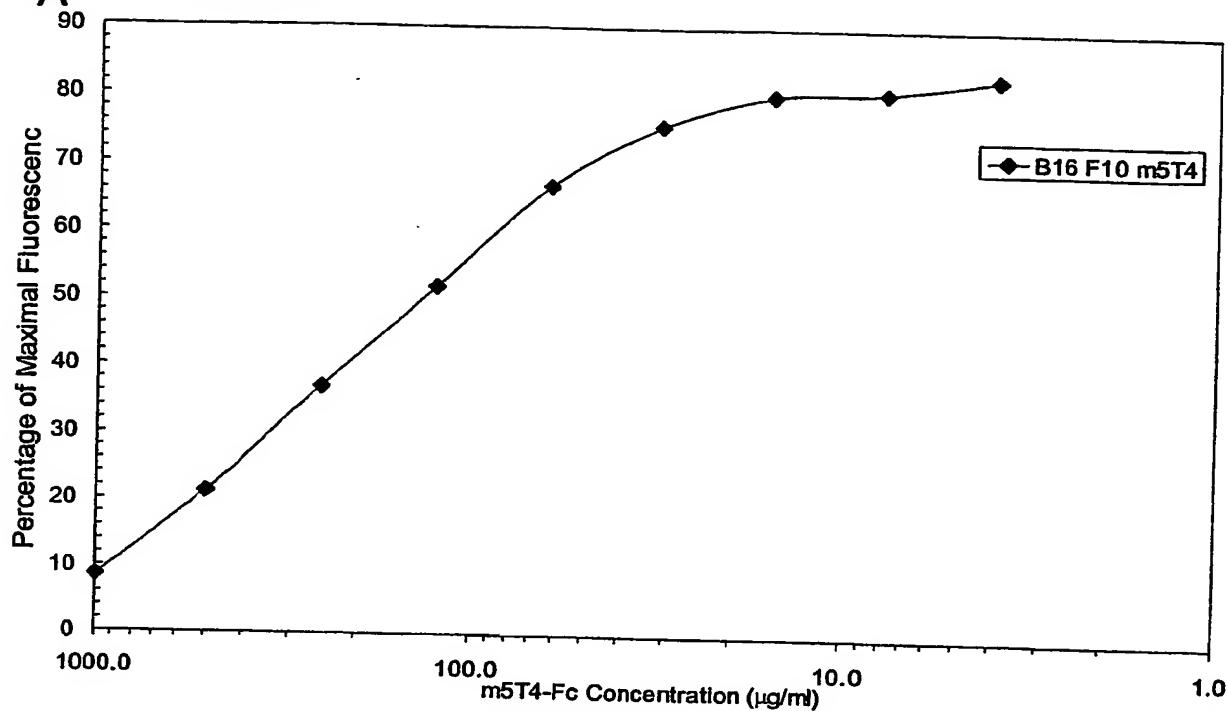
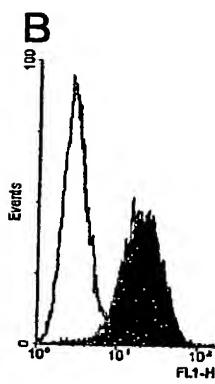
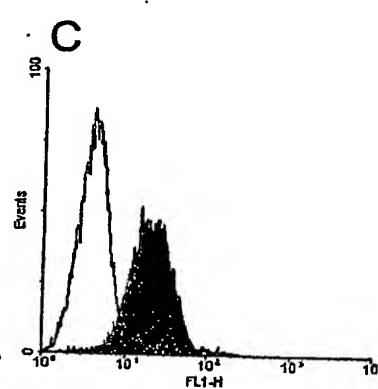
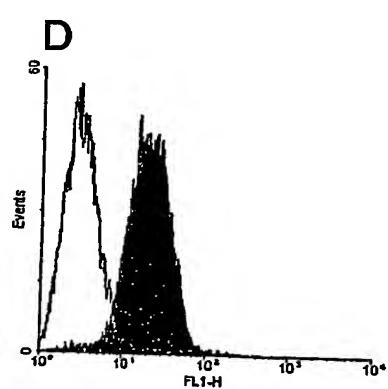
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Cell Line	Mean FSC	SD	Percentage of Neo control FSC
B16 F10-Neo	547.1	2.1	100
B16 F10-m5T4	508.9	2.1	93.00
B16 F10-h5T4	550.7	0.6	100.6
A9-H12	577.9	1.0	100
A9-m5T4	538.4	6.6	93.1
A9-h5T4	573.2	5.2	99.2
A9-mh5T4	573.4	13.6	99.2
A9-hm5T4	572.5	8.9	99.1

Table 2

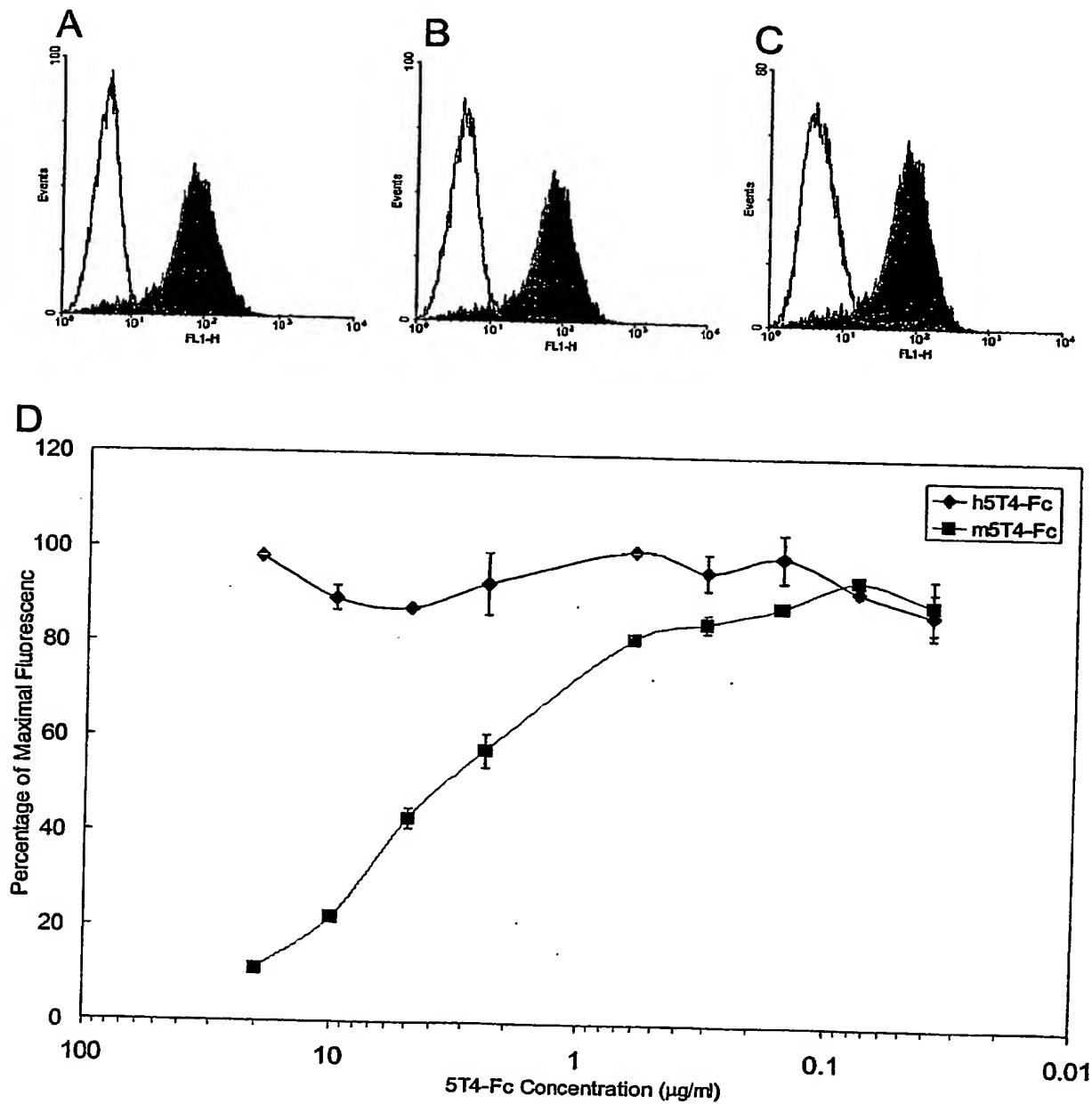
**Table 3. Common markers of ES cell differentiation.**

Marker	Method of detection	Specificity	Expression pattern following differentiation	Analysis destructive?
Alkaline phosphatase	In situ staining	ES	Negative	Y
Oct-3/4	RT-PCR	ES	Negative	Y
Rex-1	RT-PCR	ES	Negative	Y
SSEA-1	Cell-surface staining	ES	Negative	N
Forssman	Cell-surface staining	ES	Negative	N
Fgf-5	RT-PCR	Primitive ecto	Positive	Y
ZG	RT-PCR	Meso	Positive	Y
Bmp-2	RT-PCR	Endo/meso	Positive	Y
T-Bra	RT-PCR	Meso	Positive	Y
Flk-1	Cell-surface staining	Meso	Positive	N
K-18	RT-PCR	Endo/ecto	Positive	Y
Bmp-4	RT-PCR	Ecto/meso	Positive	Y
NF-68	RT-PCR	Ecto	Positive	Y
Vim	RT-PCR	Meso/endo	Positive	Y
AFP	RT-PCR	Visceral endo	Positive	Y
TTR	RT-PCR	Endo	Positive	Y

**A** Figure 1**B****C****D**

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Figure 2



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Figure 3

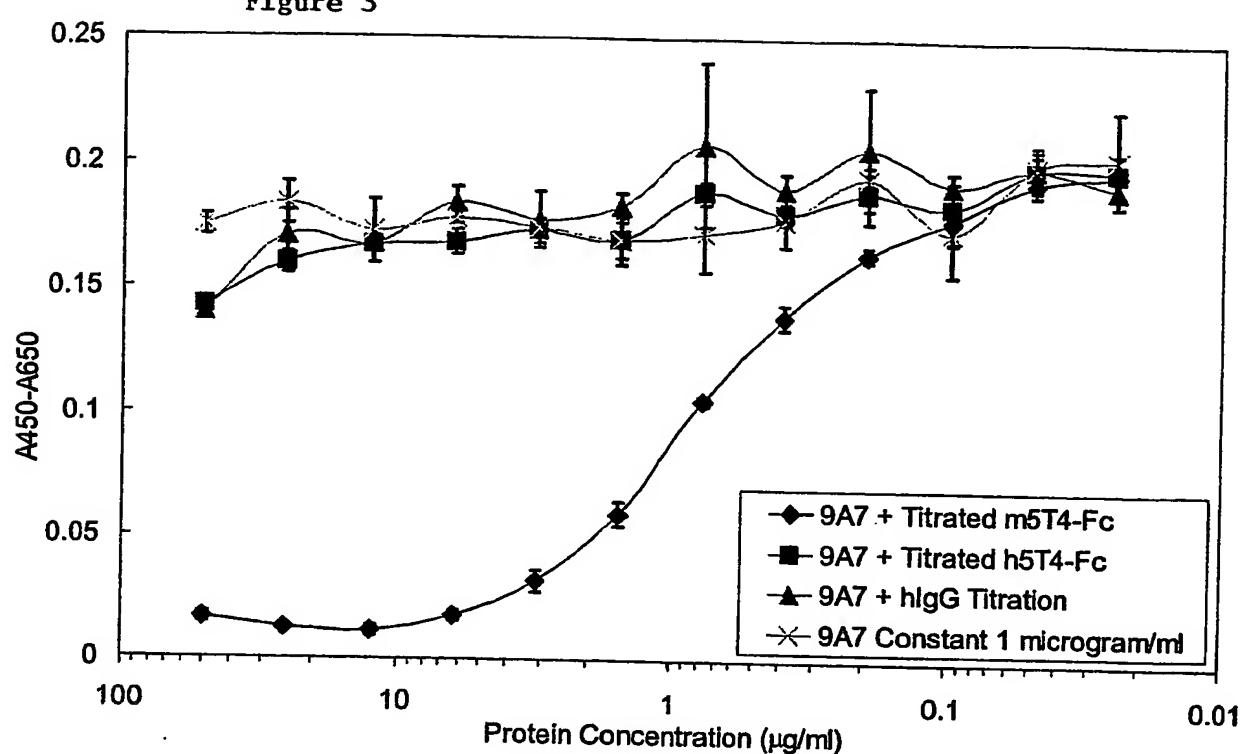


Figure 4

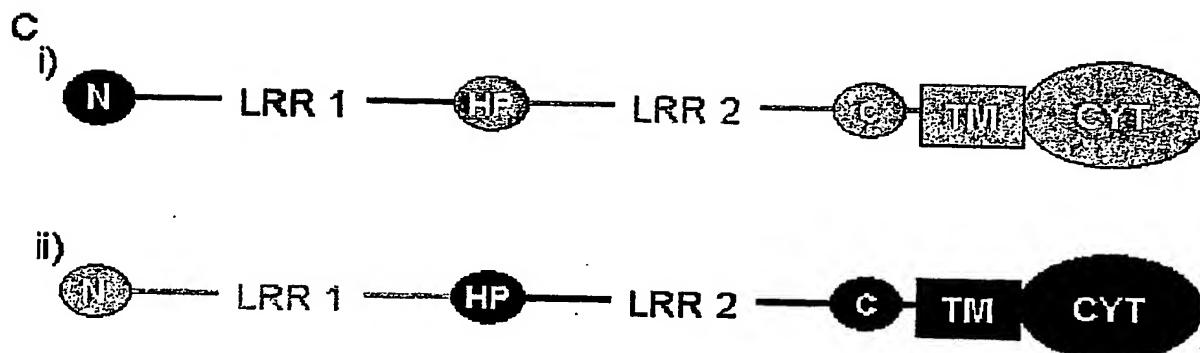
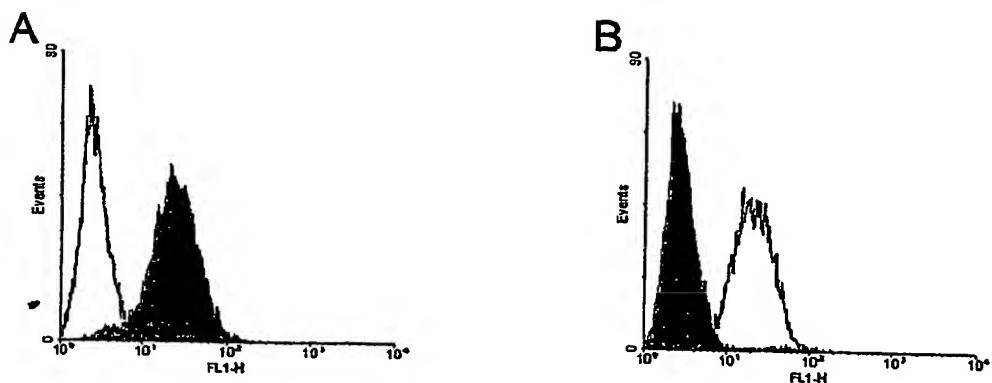
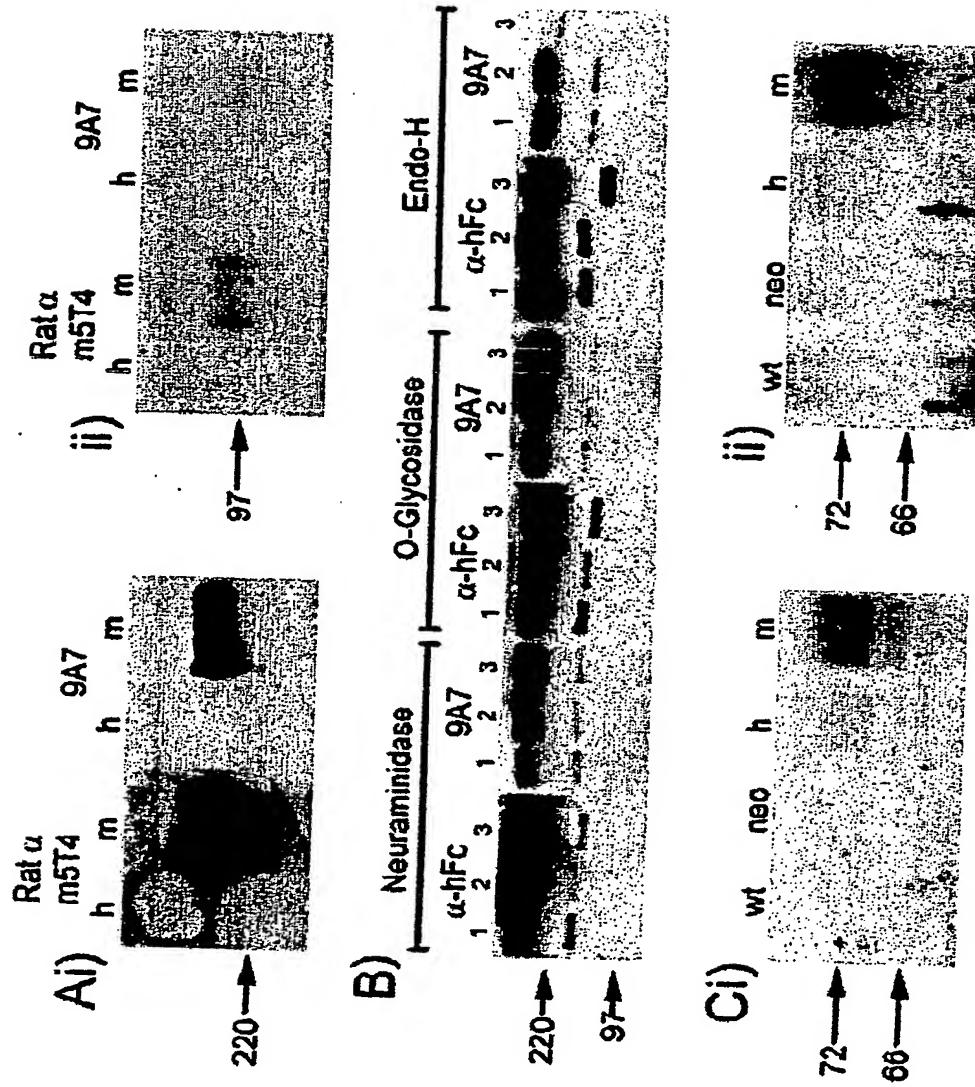
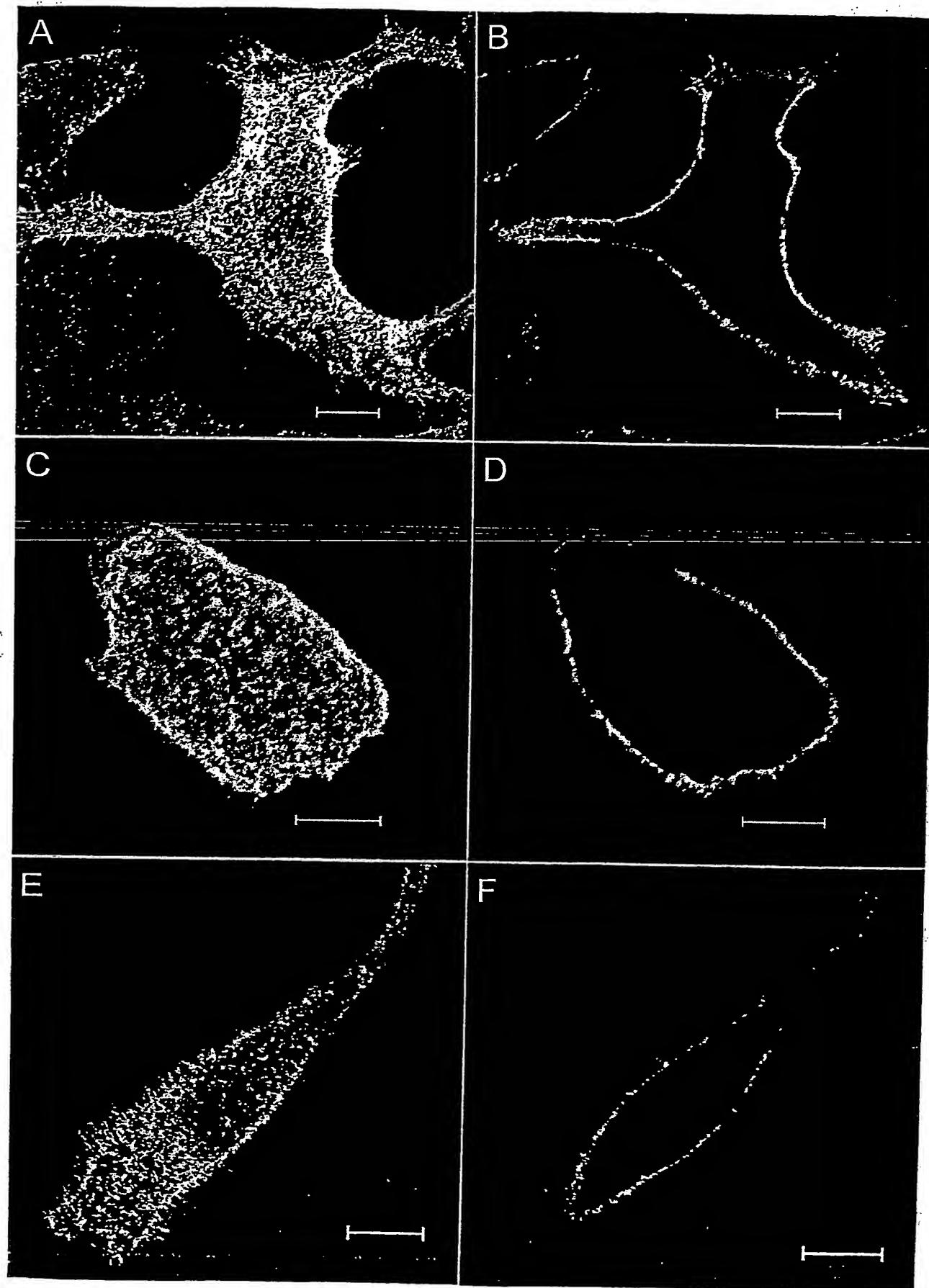


Figure 5



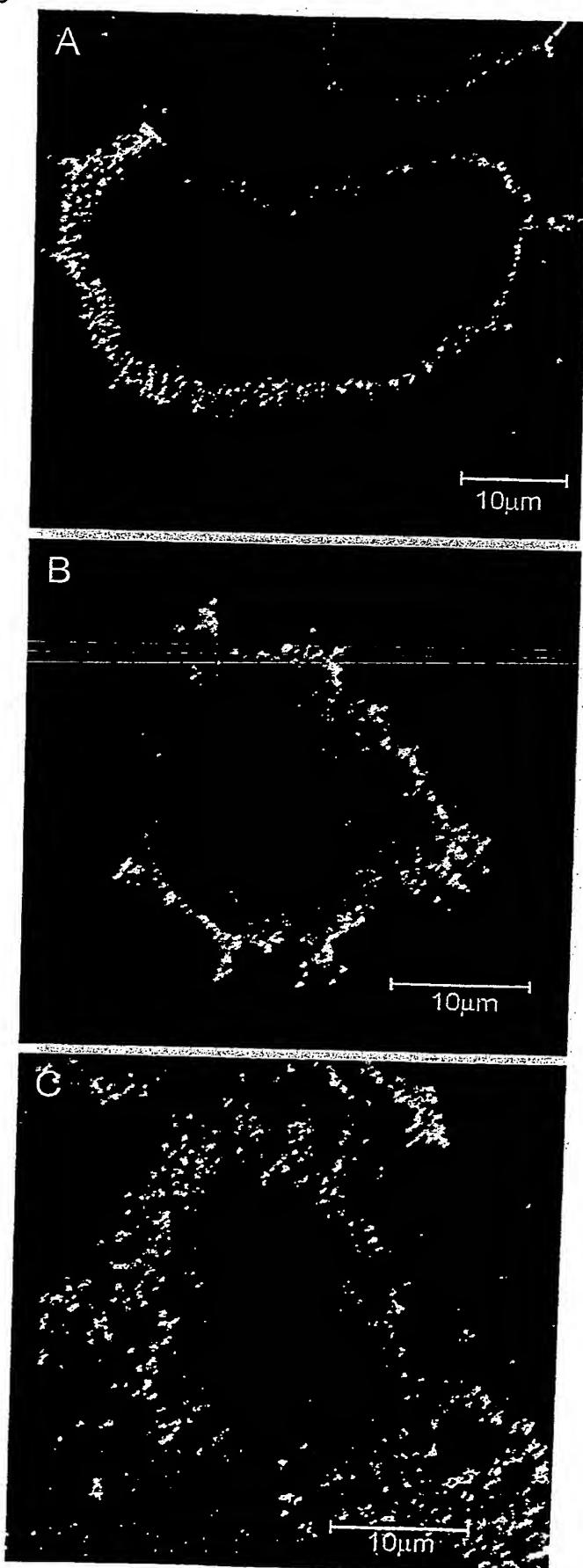
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Figure 6



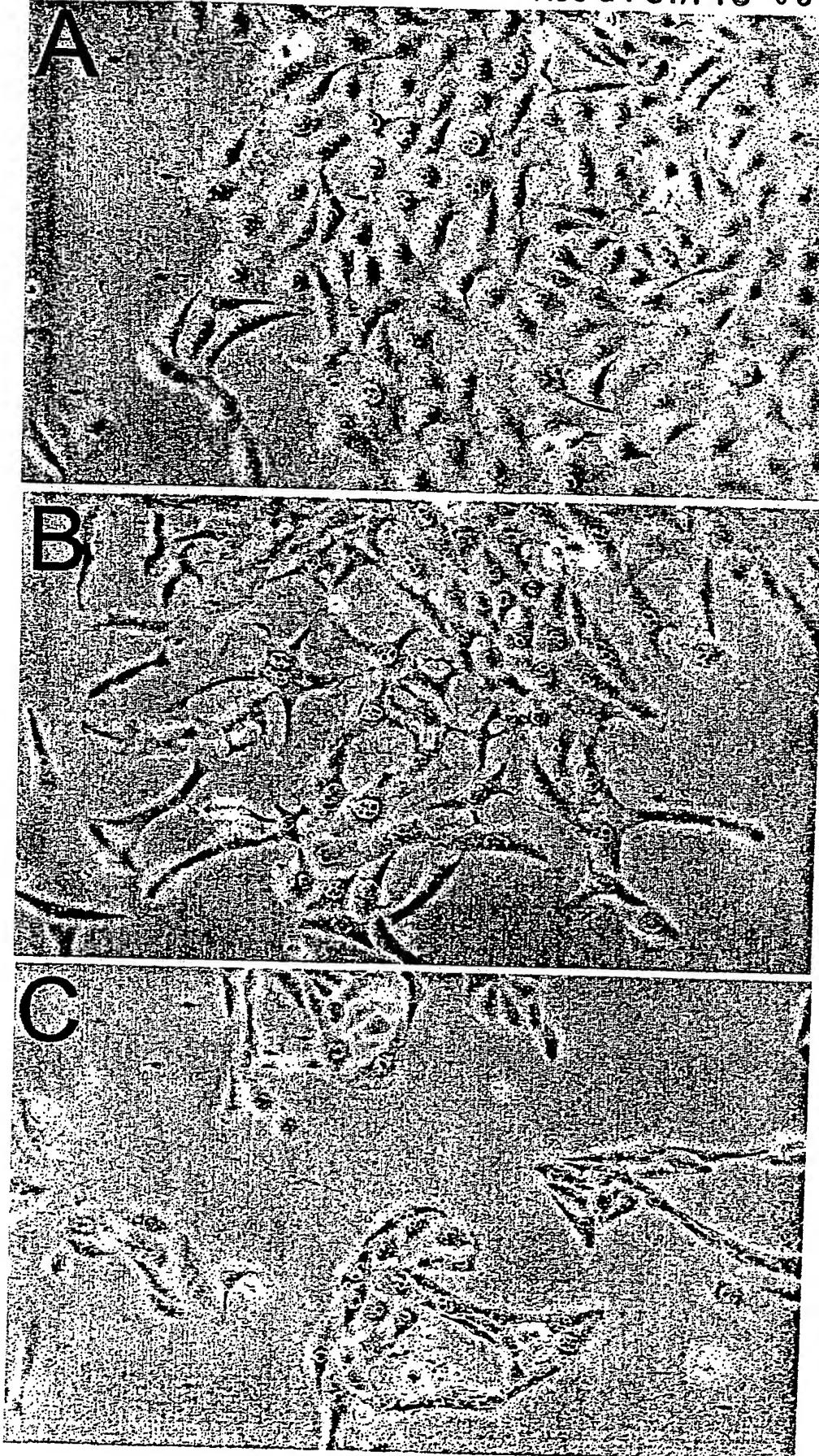
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Figure 7



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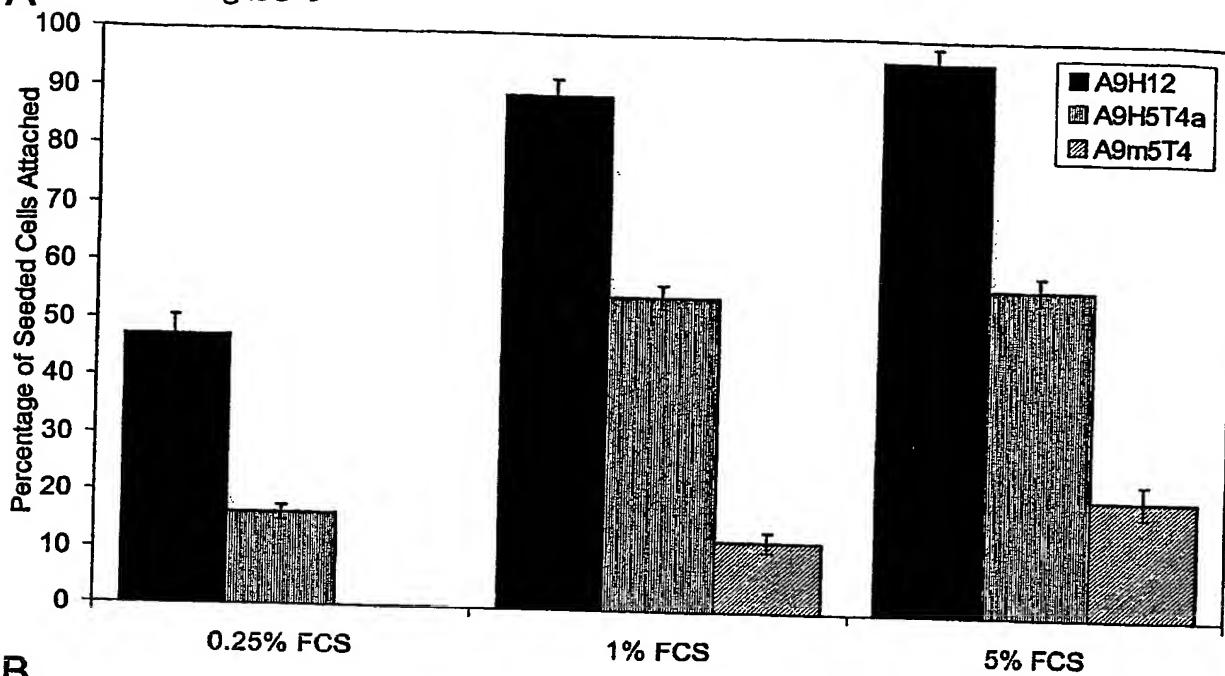
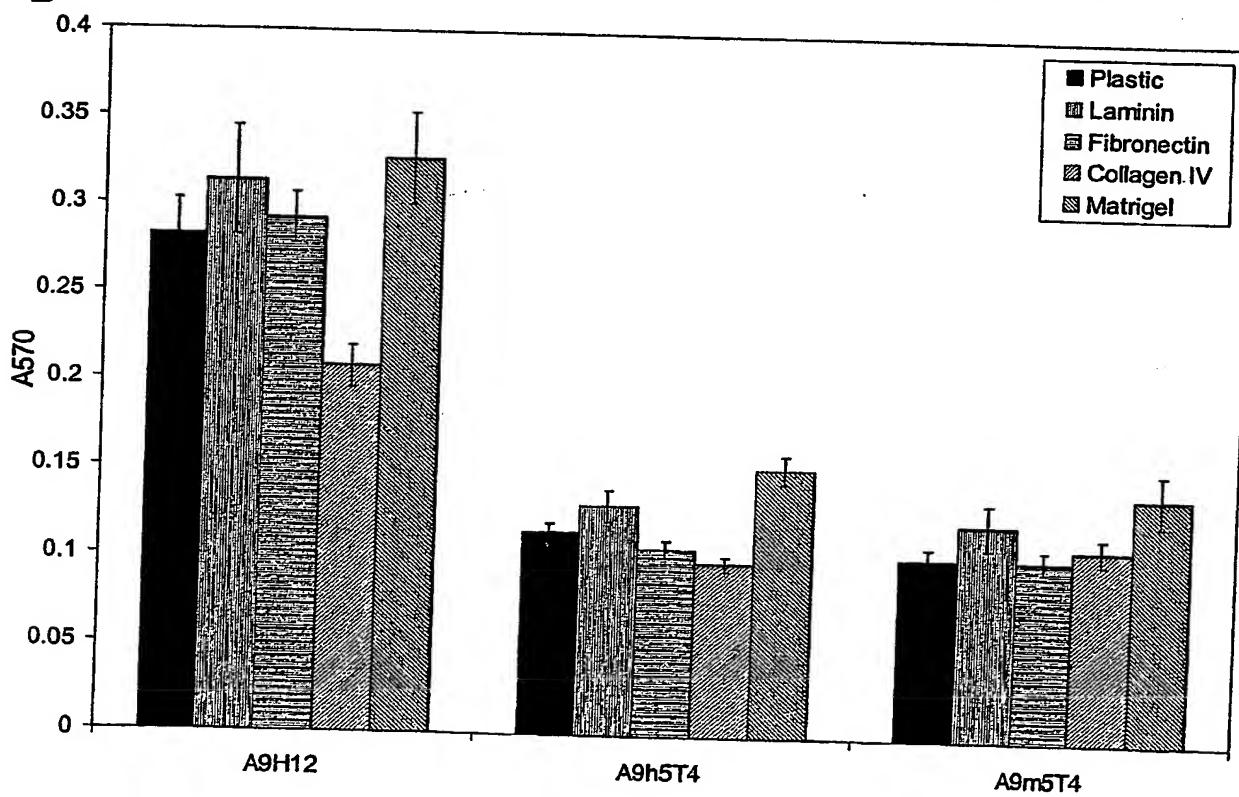
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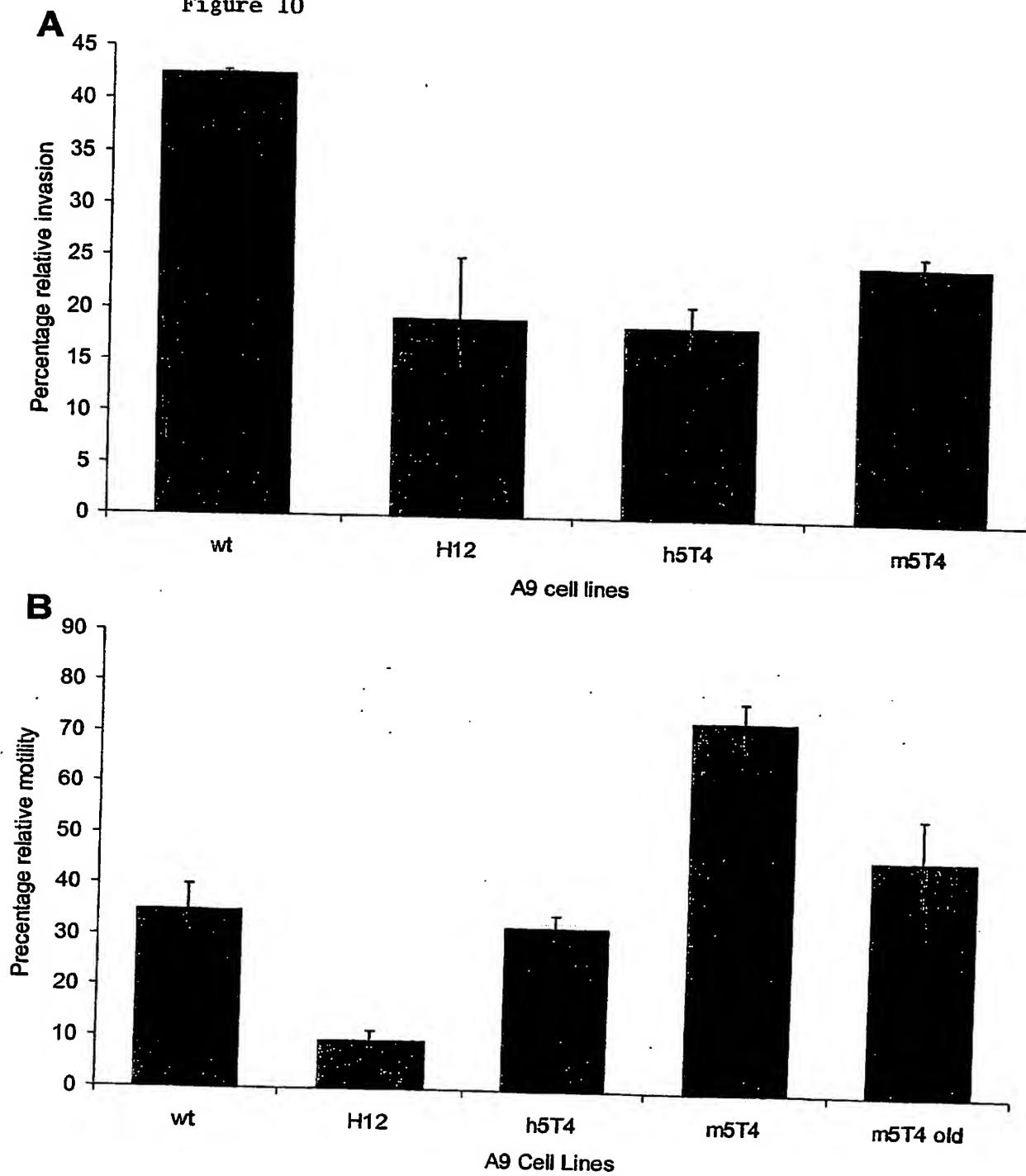
**A**

Figure 9

**B**

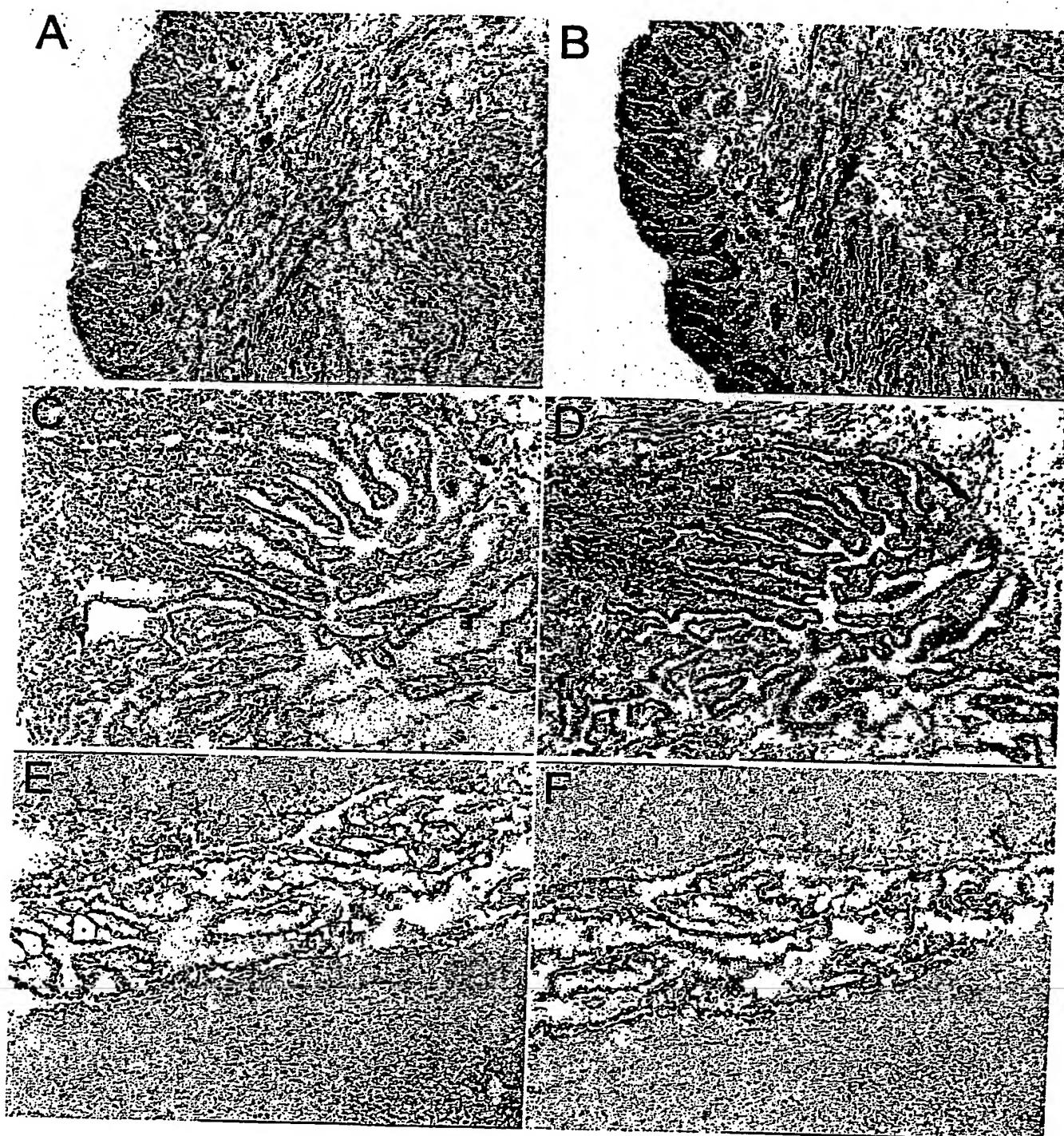
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Figure 10



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Figure 11



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Figure 12a

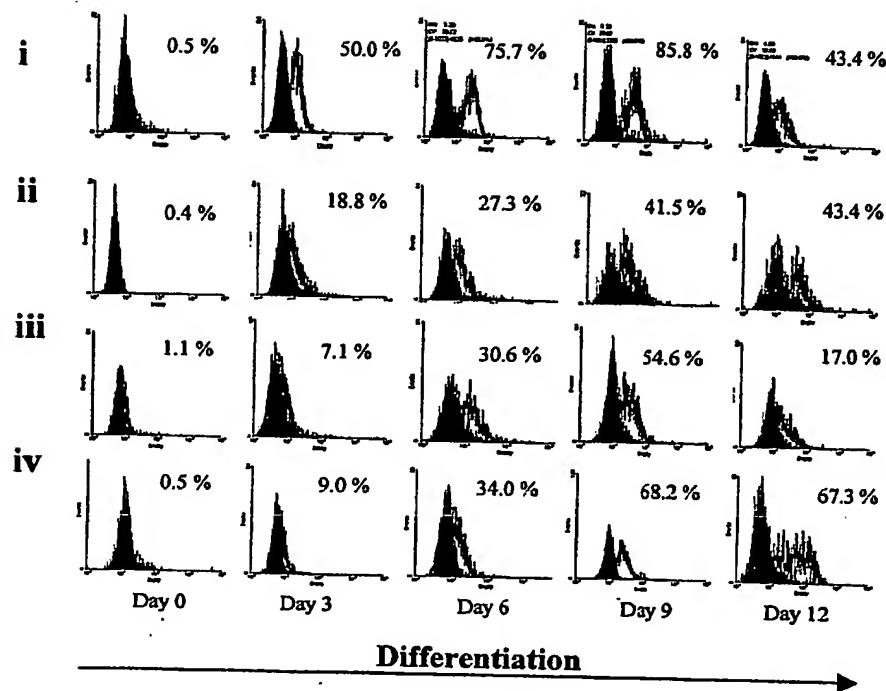
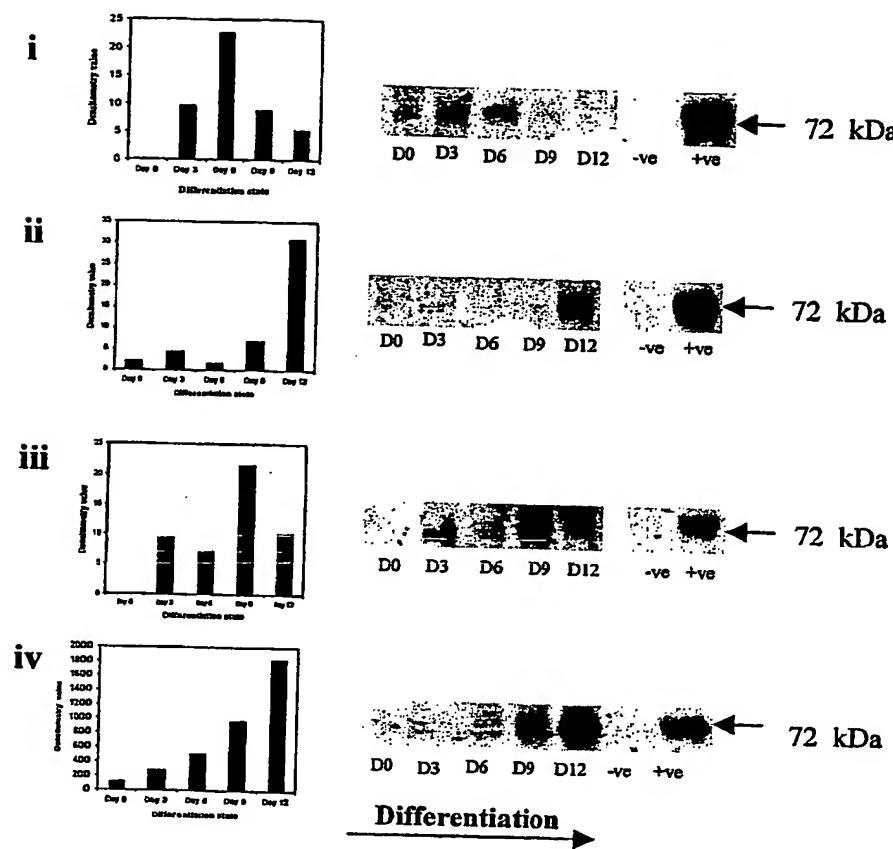


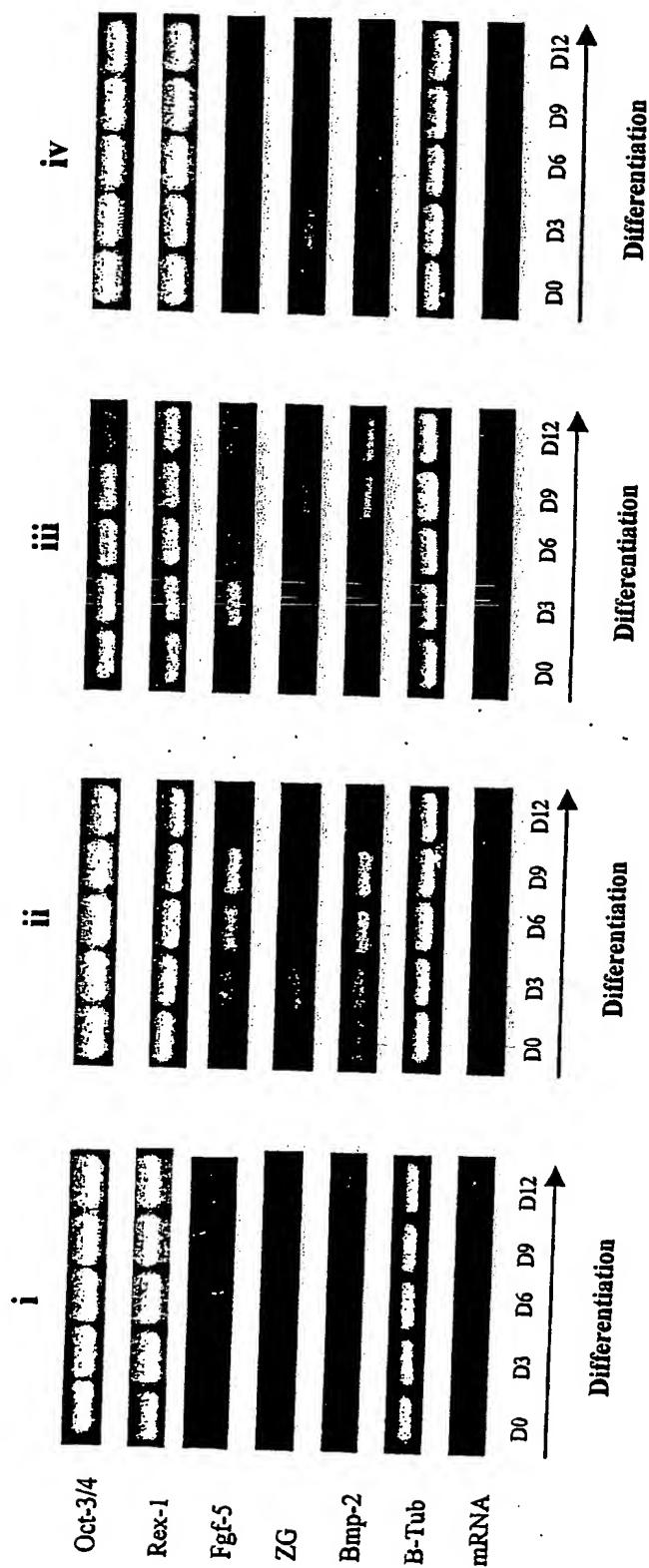
Figure 12b



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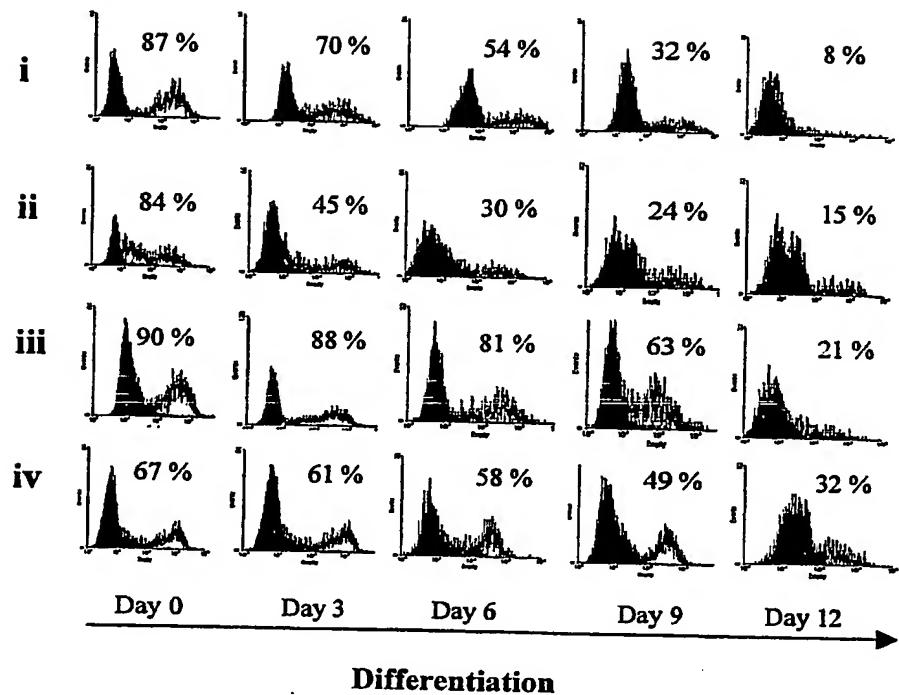
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Figure 13a



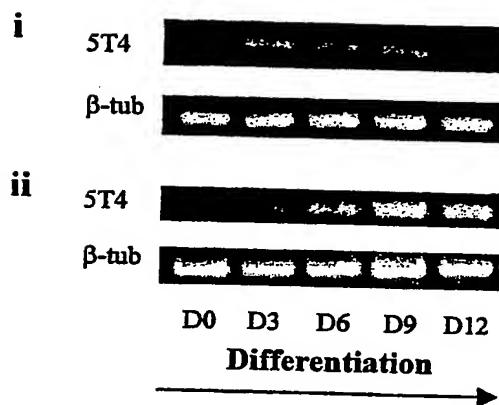
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Figure 13b



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Figure 14a



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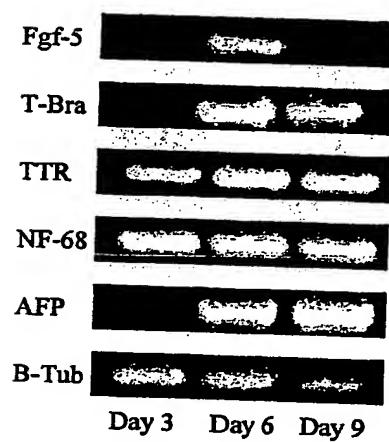
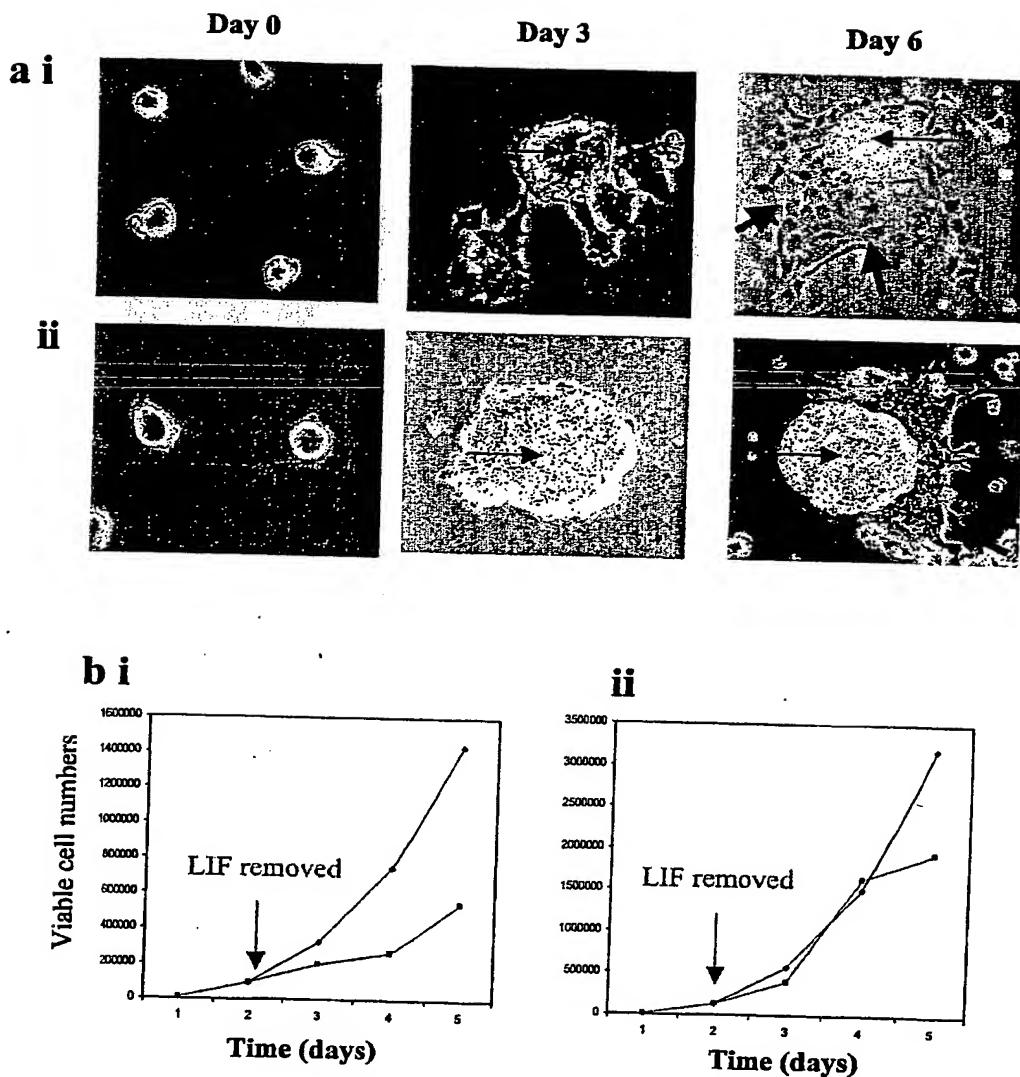
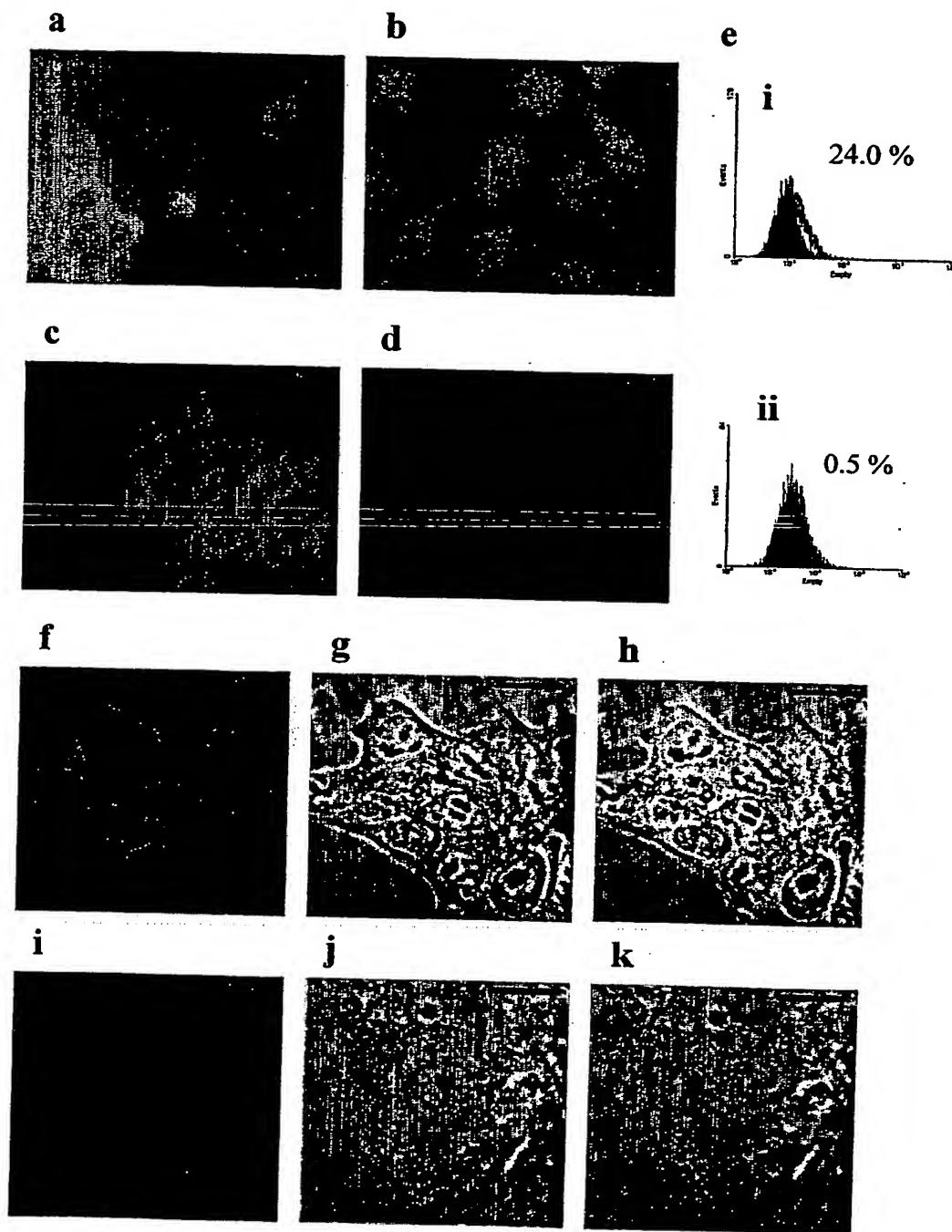
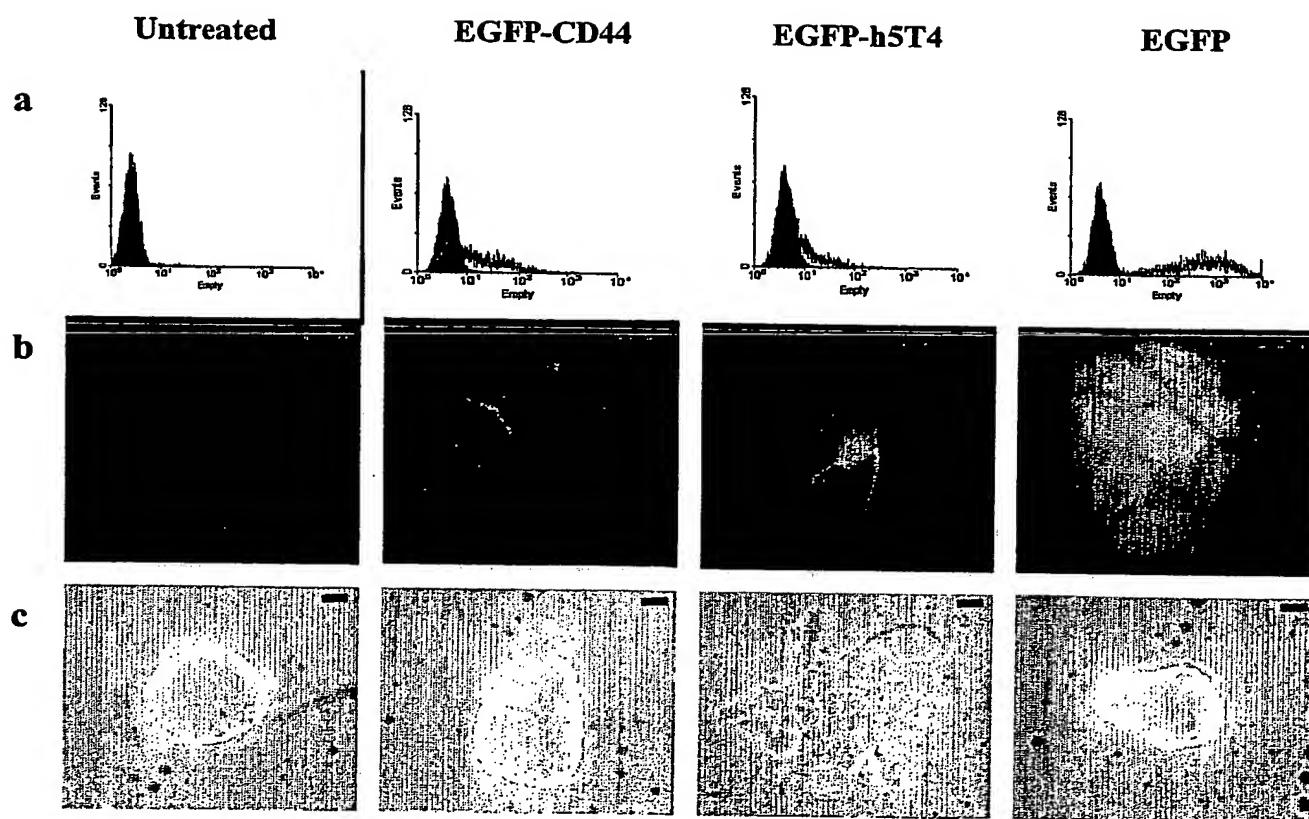
**Figure 14b**

Figure 15



**Figure 16**

**Figure 17**

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Figure 18

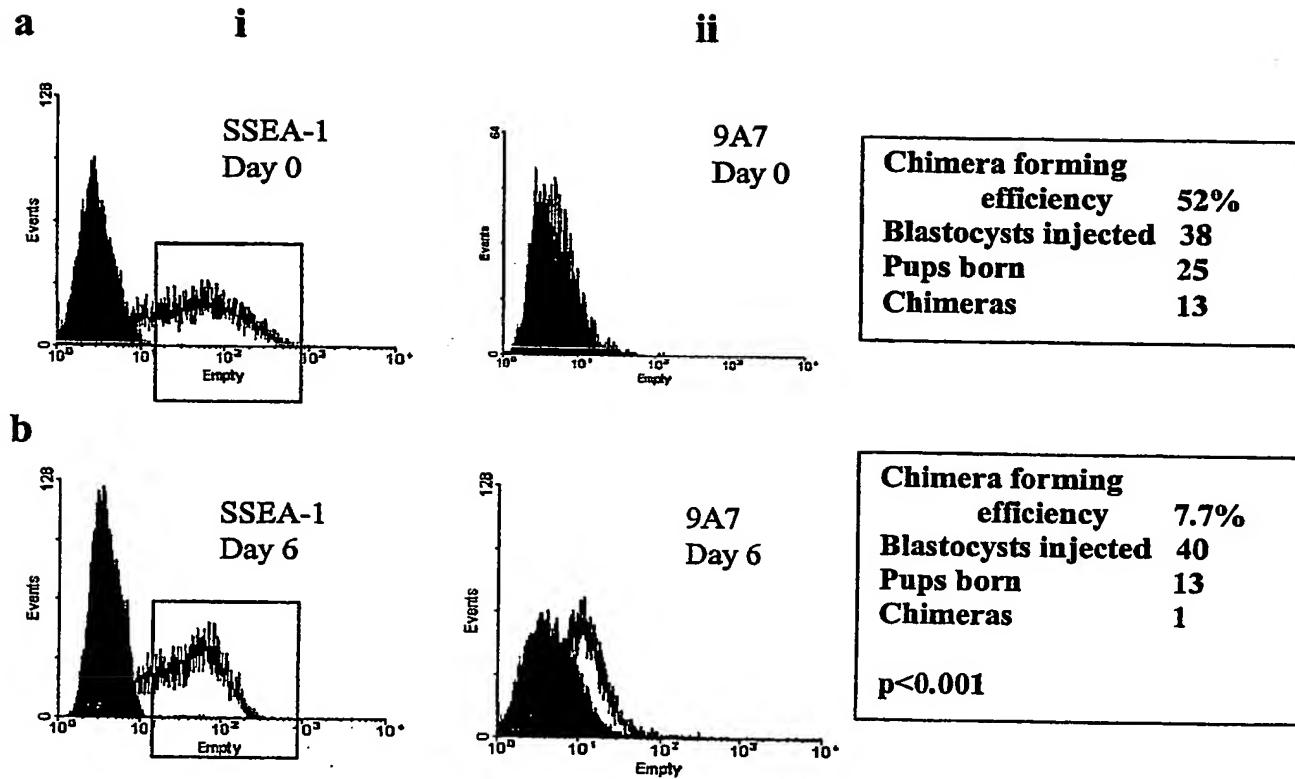
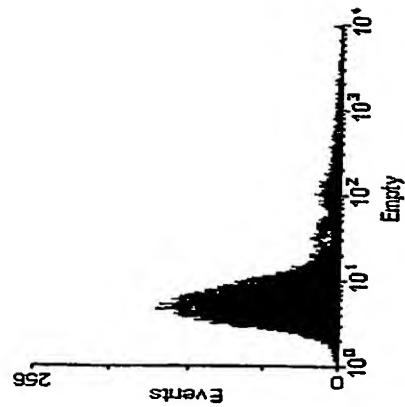
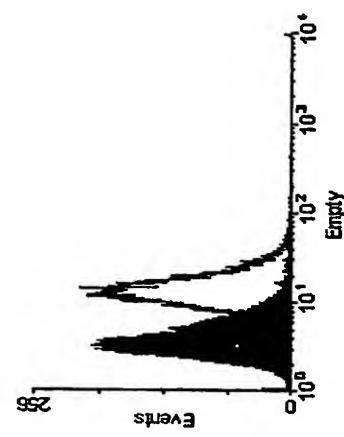


Figure 19

pefs



Tera2 clone13



+ ve control for 5T4

Figure 20  
5T4 oncofoetal antigen expression on GCT27 grown  
on pef feeders or on gelatin coated dishes.

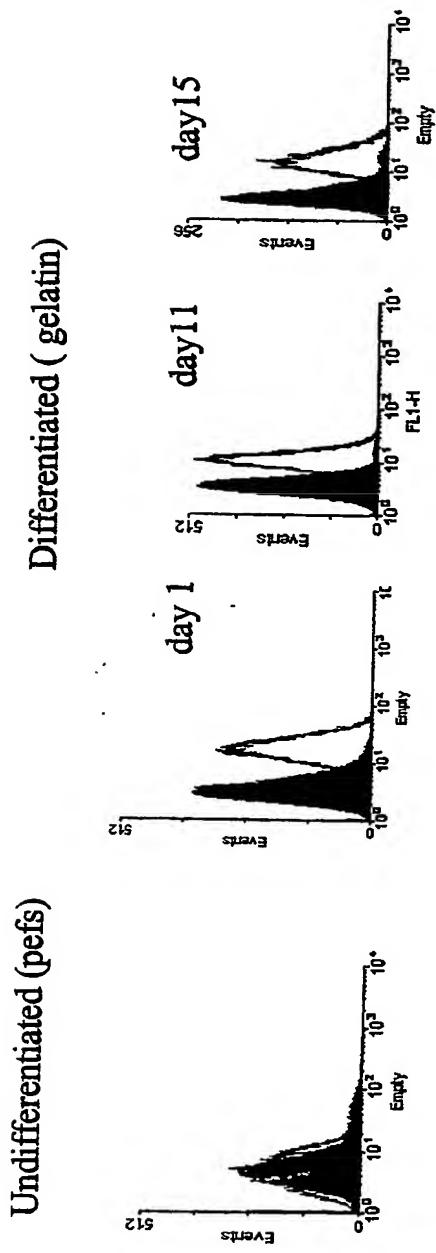
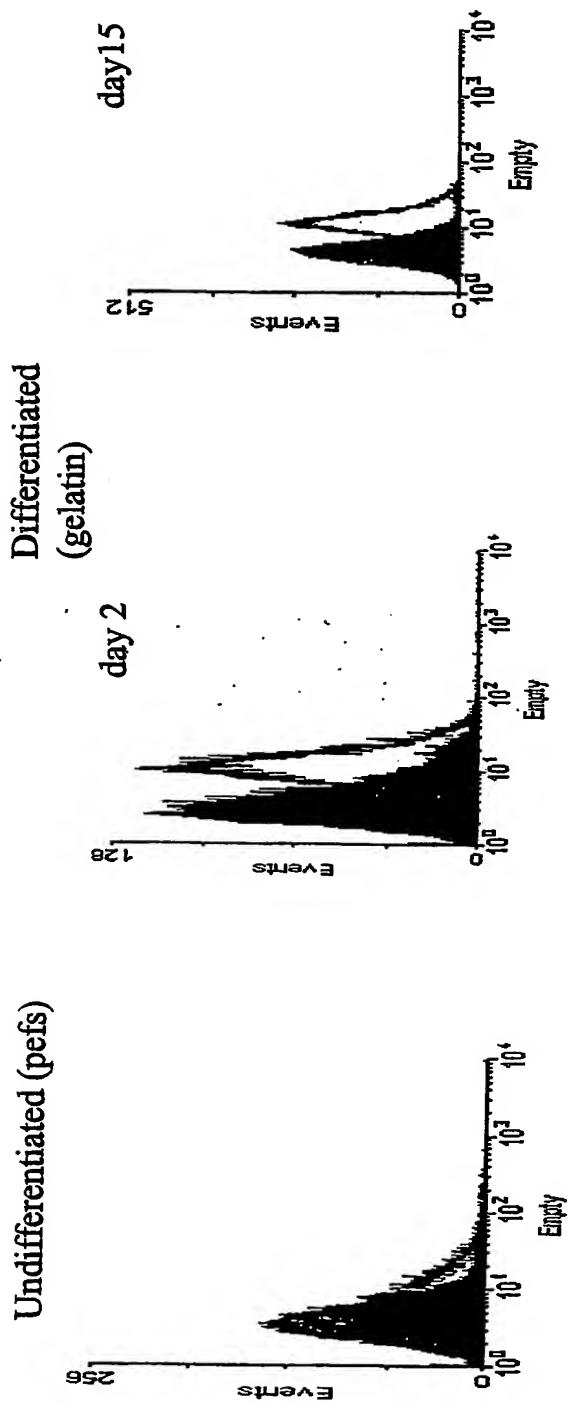


Figure 21

5T4 oncofoetal antigen expression on GCT35 grown on pef feeders or on gelatin coated dishes.

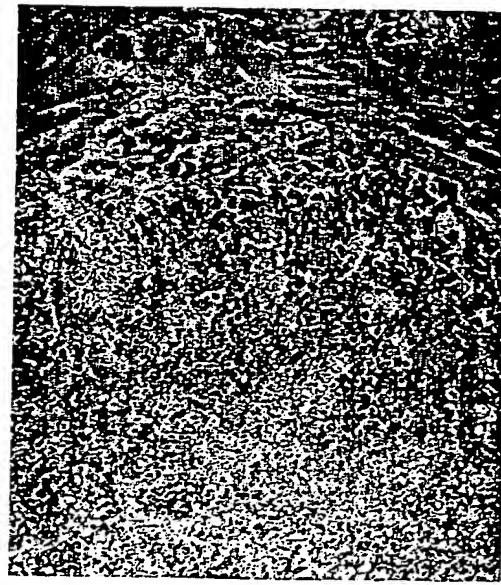


**Figure 22a**

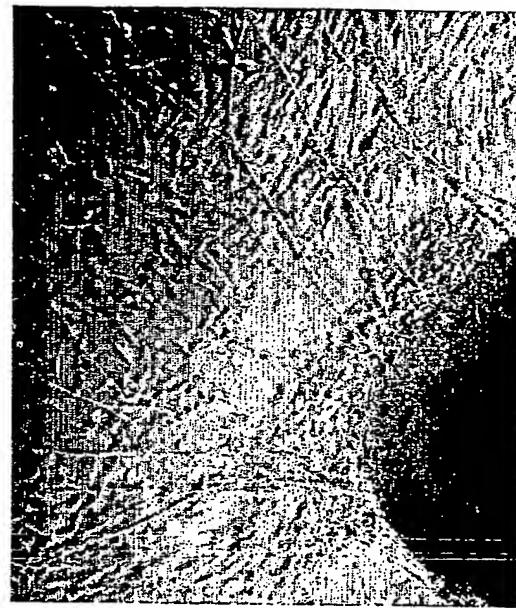
Undifferentiated ES colony on pefs  
(x100)



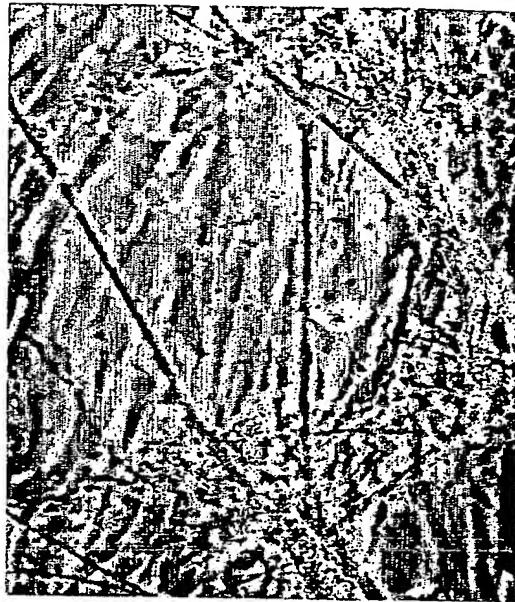
Differentiation of neural cells at the  
edge of the colony (x400)

**Figure 22b**

Differentiated ES colony (x100) on  
fibronectin coated plates and no pefs



Differentiation of neural cells at the  
edge of the colony (x400)



## Figure 23

### Expression of 5T4 in hES cells

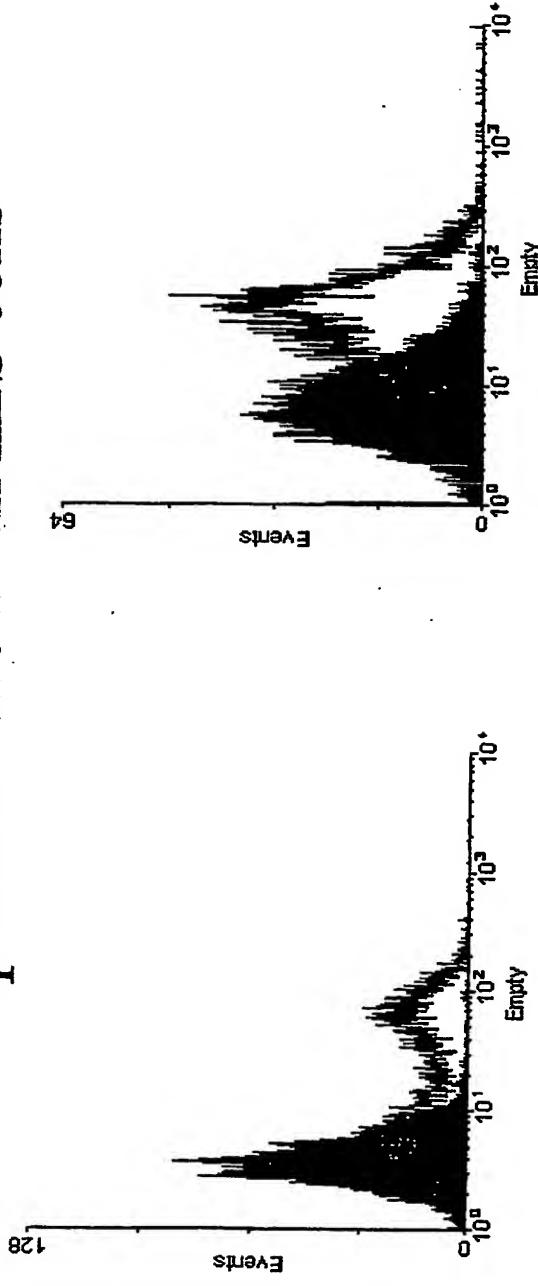
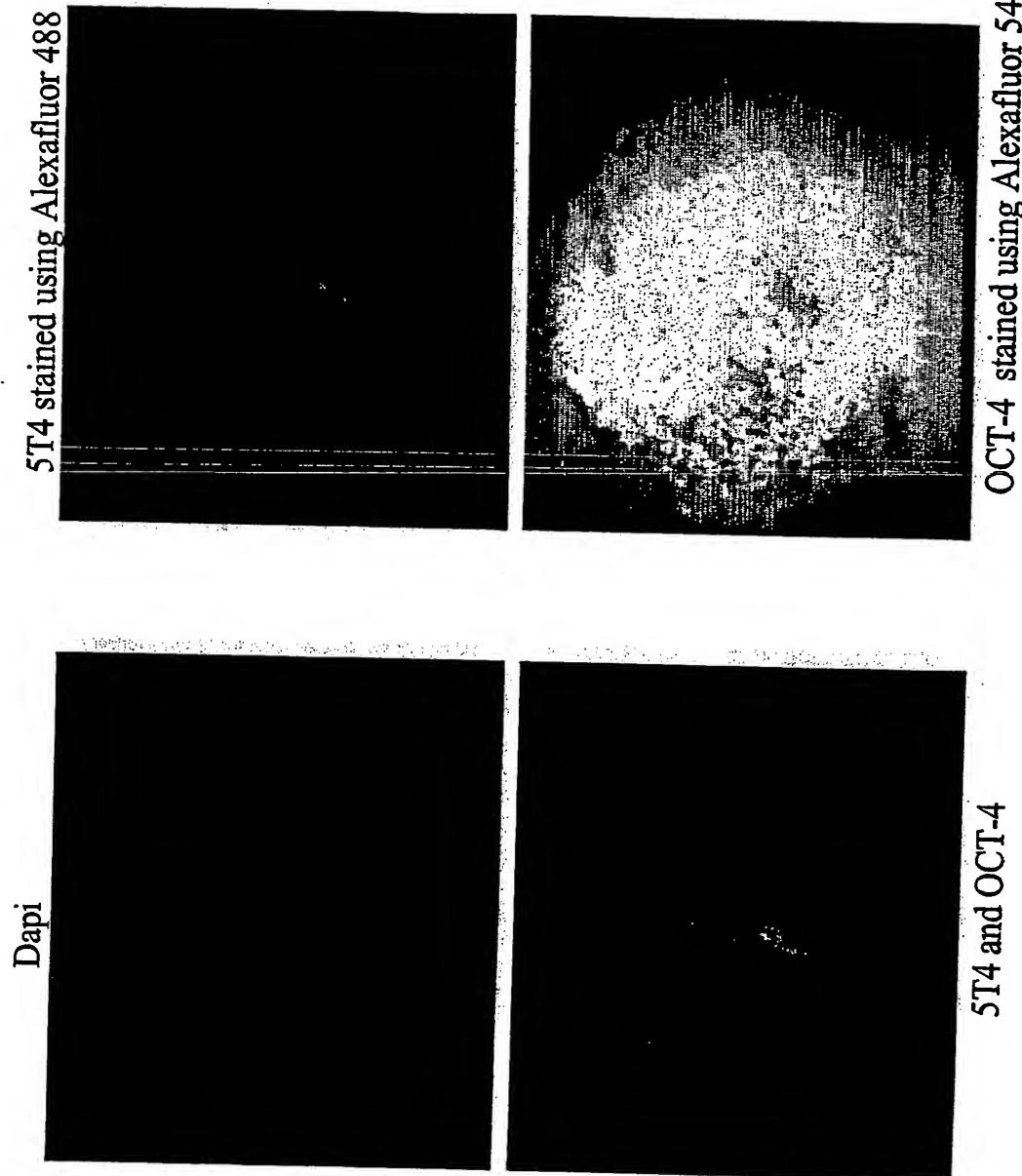


Figure 23a: "Undifferentiated" hES cells obtained from disaggregated colonies grown on pefs

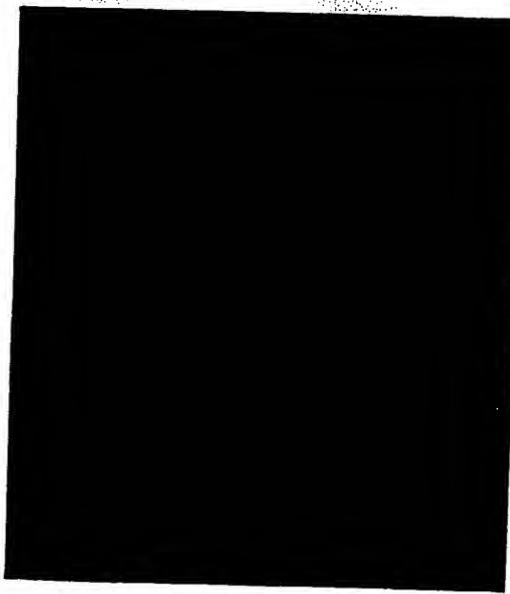
Figure 23b: Differentiated hES cells obtained from colonies grown on fibronectin coated plates and no pefs for 7 days

**Figure 24: Dual 5T4/Oct-4 staining of hES colony on pefs with a few areas of loss of Oct4 and concomitant upregulation of 5T4**

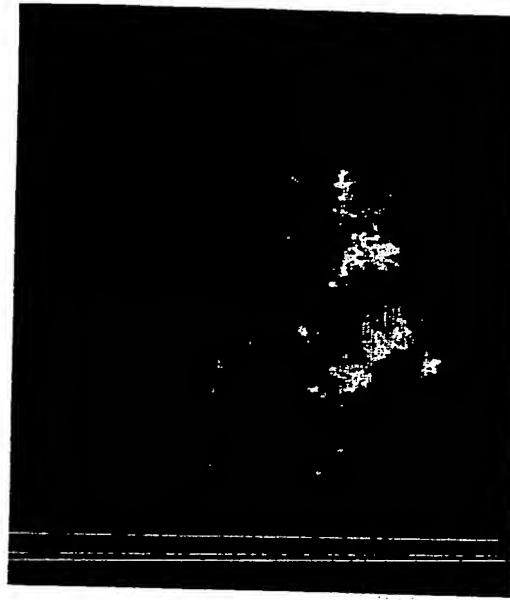


**Figure 25: Dual 5T4/Oct-4 staining of hES colony with several areas of loss of Oct4 and concomitant upregulation of 5T4**

Dapi



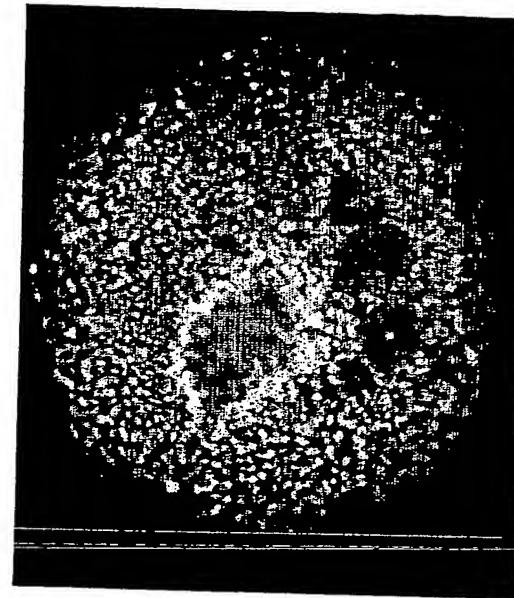
5T4 stained using Alexafluor 488



5T4 and OCT-4 overlayed



OCT-4 stained using Alexafluor 546



10/520502

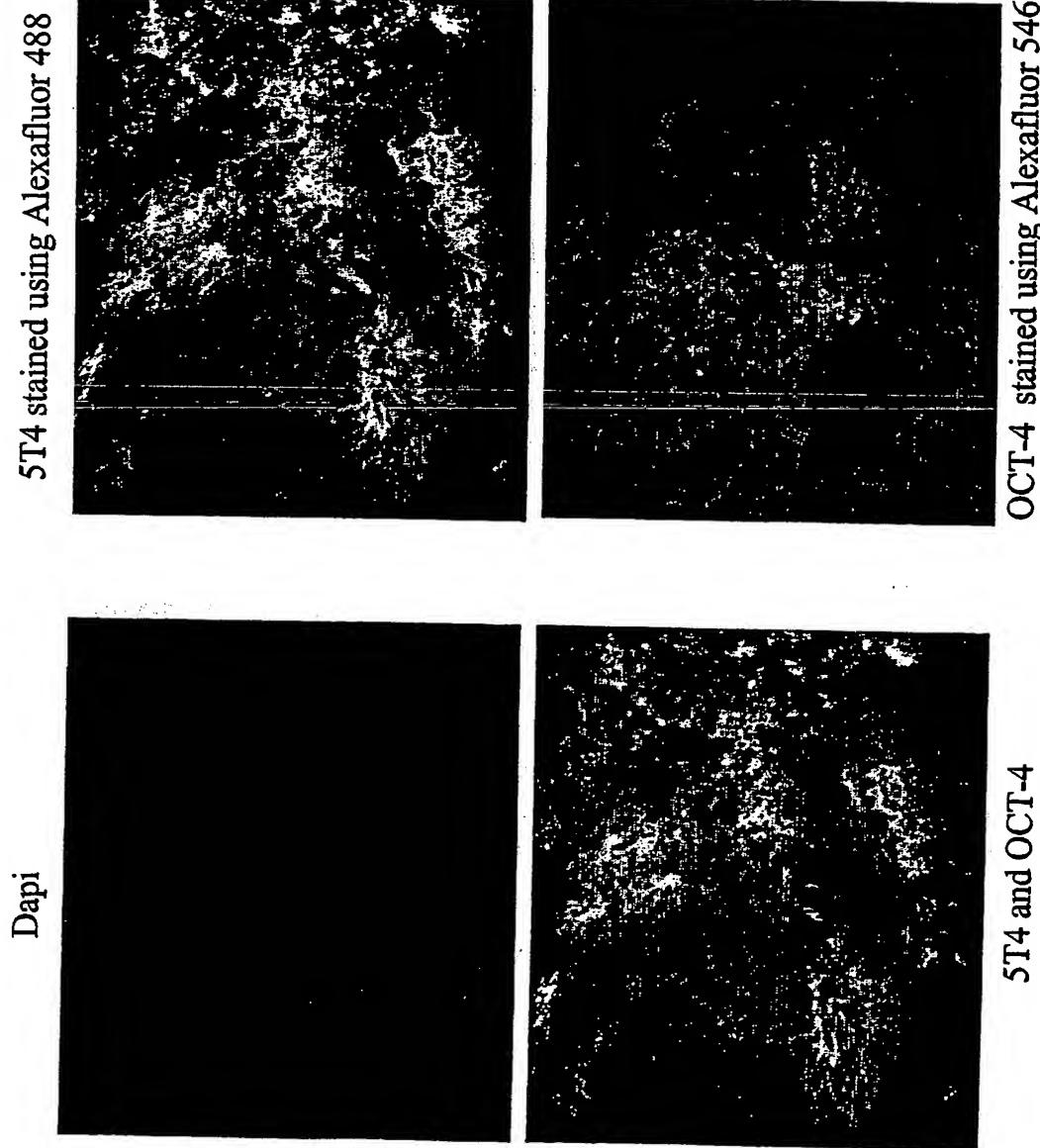
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PCT/GB2003/002836

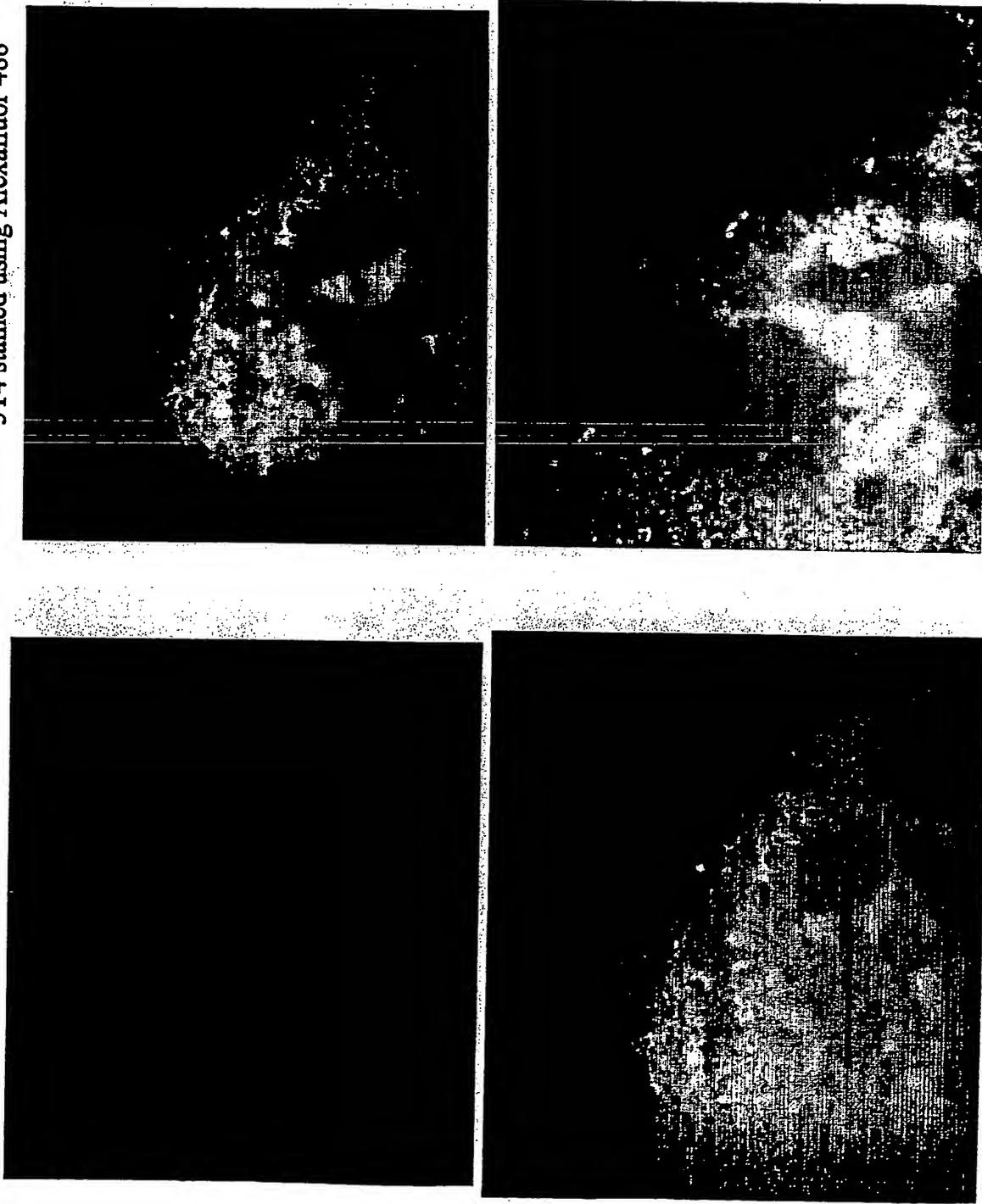
32/37

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Figure 26: Dual 5T4/Oct-4 staining of hES colony showing more extensive 5T4 expression which is mutually exclusive with OCT-4

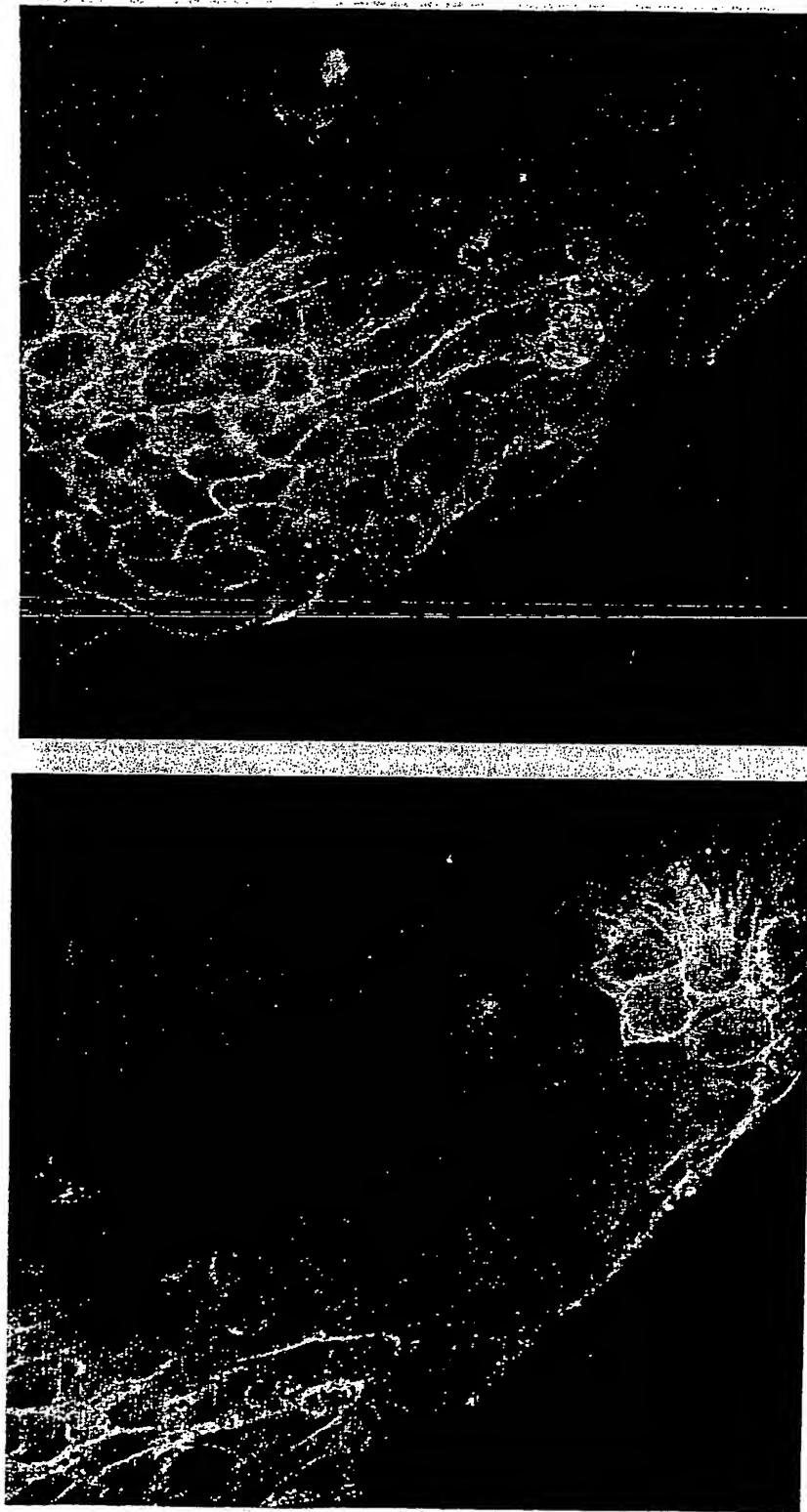


**Figure 27** Dual 5T4/Oct-4 staining of hES colony showing more extensive 5T4 expression which is mutually exclusive with OCT-4



5T4 stained using Alexafluor 488  
OCT-4 stained using Alexafluor 546  
5T4 and OCT-4

Figure 28: Confocal microscopy of dual 5T4 and OCT-4 labelling of two differentiating ES colonies.



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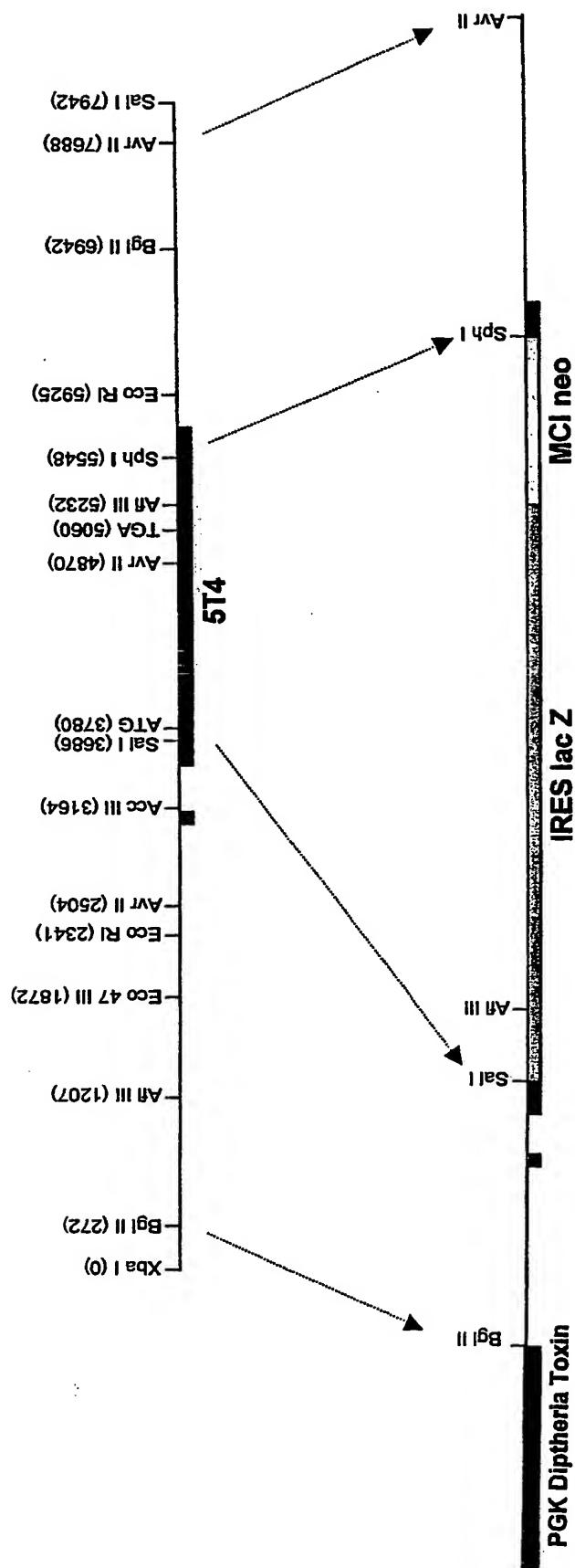
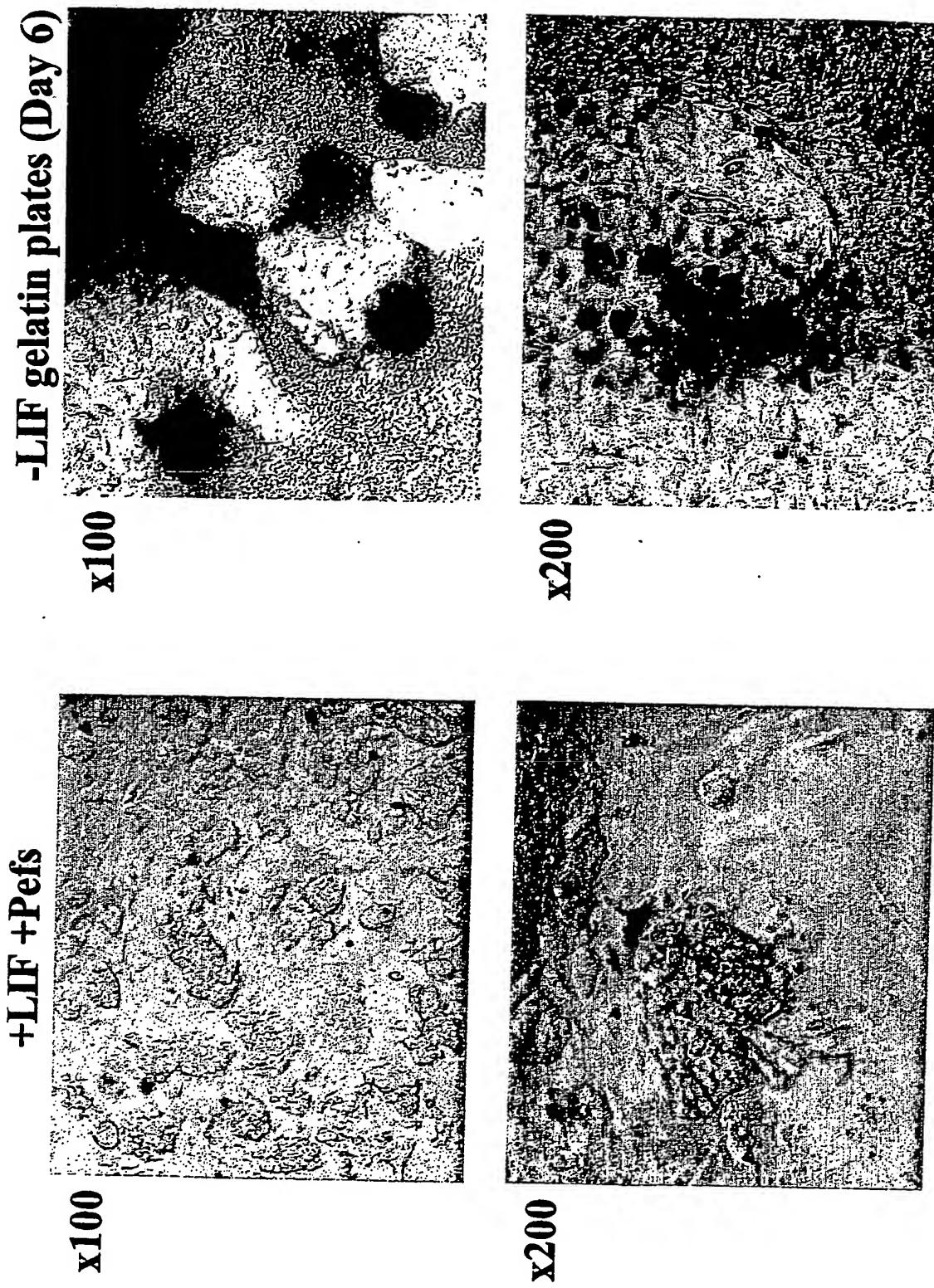


Figure 29

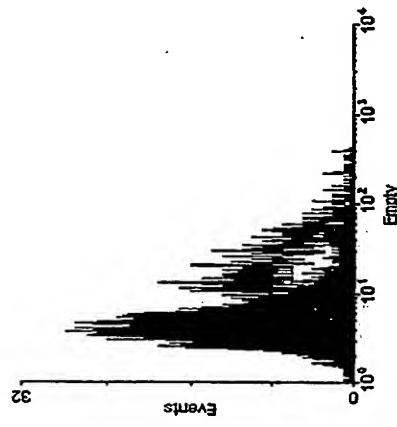
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Figure 30  $\beta$ -gal staining of undifferentiated and differentiated 5T4 KO ES cells (Clone B7)



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Figure 31: Expression of cell-surface 5T4 in MESC ES cells differentiated for 12 days as suspended embryoid bodies



Differentiation of MESC ES cells as suspended embryoid bodies was performed by transferring undifferentiated cells to bacteriological Petri dishes and subsequent growth in DMEMSR lacking LIF. The medium was changed daily.

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